abcam

Product datasheet

Anti-WSTF antibody [EP1704Y] ab51256





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Overview

Product name Anti-WSTF antibody [EP1704Y]

Description Rabbit monoclonal [EP1704Y] to WSTF

Host species Rabbit

Tested applications Suitable for: IHC-P, WB, Flow Cyt (Intra), ICC/IF

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide within Human WSTF (C terminal). The exact sequence is proprietary.

Positive control WB: Wild-type HAP1, HeLa (ab150035), and PC12 cell lysates. Mouse and Rat testis. B16-F0,

MCF7, HeLa whole cell lysates. Flow cyt: HeLa cells; IHC: rat cardiac muscle tissue, mouse

cardiac muscle tissue, human breast carcinoma; ICC/IF: HeLa cells.

General notes This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity - Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol (glycerin, glycerine), 59% PBS, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EP1704Y

Isotype lgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab51256 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P		1/700. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB	★★★★☆ (2)	1/15000 - 1/20000. Detects a band of approximately 185 kDa (predicted molecular weight: 171 kDa).
Flow Cyt (Intra)		1/20. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/50. For unpurified format use at 1/100 to 1/250 dilution

Target

Function

Atypical tyrosine-protein kinase that plays a central role in chromatin remodeling and acts as a transcription regulator. Involved in DNA damage response by phosphorylating 'Tyr-142' of histone H2AX (H2AXY142ph). H2AXY142ph plays a central role in DNA repair and acts as a mark that distinguishes between apoptotic and repair responses to genotoxic stress. Essential component of the WICH complex, a chromatin remodeling complex that mobilizes nucleosomes and reconfigures irregular chromatin to a regular nucleosomal array structure. The WICH complex regulates the transcription of various genes, has a role in RNA polymerase I and RNA polymerase III transcription, mediates the histone H2AX phosphorylation at 'Tyr-142', and is involved in the maintenance of chromatin structures during DNA replication processes. In the complex, it mediates the recruitment of the WICH complex to replication foci during DNA replication. Also involved in vitamin D-coupled transcription regulation via its association with the WINAC complex, a chromatin-remodeling complex recruited by vitamin D receptor (VDR), which is required for the ligand-bound VDR-mediated transrepression of the CYP27B1 gene. In the WINAC complex, plays an essential role by targeting the complex to acetylated histones, an essential step for VDR-promoter association.

Tissue specificity

Ubiquitously expressed with high levels of expression in heart, brain, placenta, skeletal muscle and ovary.

Involvement in disease

Note=BAZ1B is located in the Williams-Beuren syndrome (WBS) critical region. WBS results from a hemizygous deletion of several genes on chromosome 7q11.23, thought to arise as a consequence of unequal crossing over between highly homologous low-copy repeat sequences flanking the deleted region. Haploinsufficiency of BAZ1B may be the cause of certain cardiovascular and musculo-skeletal abnormalities observed in the disease.

Sequence similarities

Belongs to the WAL family. BAZ1B subfamily.

Contains 1 bromo domain.
Contains 1 DDT domain.

Contains 1 PHD-type zinc finger.

Contains 1 WAC domain.

Developmental stage

Expressed at equal levels in 19-23 weeks old fetal tissues.

Domain

The N-terminal part (1-345), including the WAC domain and the C motif, mediates the tyrosine-

protein kinase activity.

The bromo domain mediates the specific interaction with acetylated histones.

Post-translational modifications

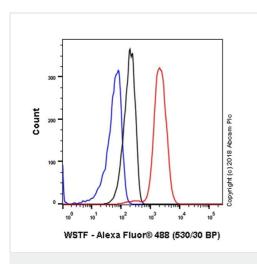
Phosphorylated upon DNA damage, probably by ATM or ATR.

Cellular localization

Nucleus. Accumulates in pericentromeric heterochromatin during replication. Targeted to

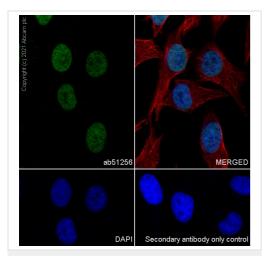
replication foci throughout S phase via its association with PCNA.

Images

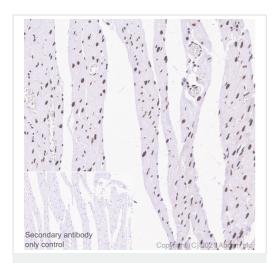


Flow Cytometry (Intracellular) - Anti-WSTF antibody [EP1704Y] (ab51256)

Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling WSTF with Purified ab51256 at 1:20 dilution (10 µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit lgG (Alexa Fluor® 488, <u>ab150077</u>) secondary antibody was used at 1:2000. Isotype control - Rabbit monoclonal lgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunocytochemistry/ Immunofluorescence - Anti-WSTF antibody [EP1704Y] (ab51256) Immunocytochemistry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling WSTF with Purified ab51256 at 1:50 dilution (2.9 μ g/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 μ g/ml). Goat anti rabbit lgG (Alexa Fluor® 488, <u>ab150077</u>) was used as the secondary antibody at 1:1000 (2 μ g/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



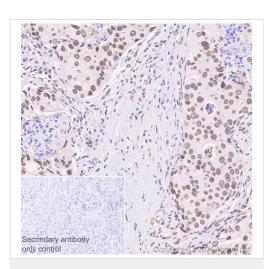
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-WSTF antibody
[EP1704Y] (ab51256)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat cardiac muscle tissue sections labeling WSTF with Purified ab51256 at 1:700 dilution (0.211 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using **ab93678** (citrate buffer, pH 6.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.



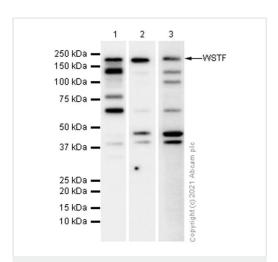
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-WSTF antibody
[EP1704Y] (ab51256)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse cardiac muscle tissue sections labeling WSTF with Purified ab51256 at 1:700 dilution (0.211 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using ab93678 (citrate buffer, pH 6.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-WSTF antibody
[EP1704Y] (ab51256)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue sections labeling WSTF with Purified ab51256 at 1:700 dilution (0.211 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using ab93678 (citrate buffer, pH 6.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.



Western blot - Anti-WSTF antibody [EP1704Y] (ab51256)

All lanes : Anti-WSTF antibody [EP1704Y] (ab51256) at 1/5000 dilution

Lane 1: B16-F0 (Mouse melanoma epithelial cell-like) whole cell

lysate

Lane 2 : Mouse testis lysate

Lane 3: Rat testis lysate

Lysates/proteins at 15 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

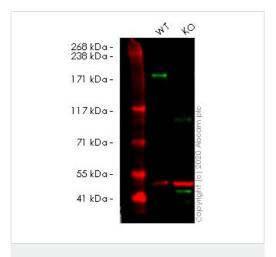
Predicted band size: 171 kDa **Observed band size:** 185 kDa

Exposure time

Lane 1: 10 seconds

Lane 2: 40 seconds

Lane 3: 180 seconds



Western blot - Anti-WSTF antibody [EP1704Y] (ab51256)

All lanes: Anti-WSTF antibody [EP1704Y] (ab51256) at 1/15000

dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: BAZ1B knockout HeLa cell lysate

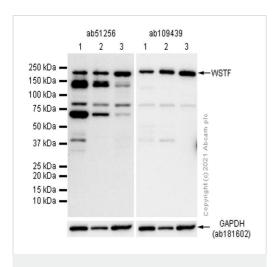
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 171 kDa **Observed band size:** 175 kDa

Lanes 1-2: Merged signal (red and green). Green - ab51256 observed at 175 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) observed at 50 kDa.

ab51256 was shown to react with WSTF in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab264907 (knockout cell lysate ab257370) was used. Wild-type HeLa and WSTF knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab51256 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) overnight at 4°C at a 1 in 15000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-WSTF antibody [EP1704Y] (ab51256)

All lanes : Anti-WSTF antibody [EP1704Y] (ab51256) at 1/20000 dilution

Lane 1: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 2 : T-47D (Human ductal breast epithelial tumor epithelial cell) whole cell lysate

Lane 3 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated)

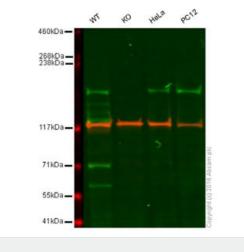
Predicted band size: 171 kDa

Exposure time:

Left image: 180 seconds

Right image: 40 seconds

We recommend to use <u>ab109439</u> for WSTF Western Blot testing because ab51256 detects nonspecific bands.



Western blot - Anti-WSTF antibody [EP1704Y] (ab51256)

All lanes : Anti-WSTF antibody [EP1704Y] (ab51256) at 1/15000 dilution

Lane 1: Wild-type HAP1 cell lysate

Lane 2: WSTF knockout HAP1 cell lysate

Lane 3 : HeLa cell lysate

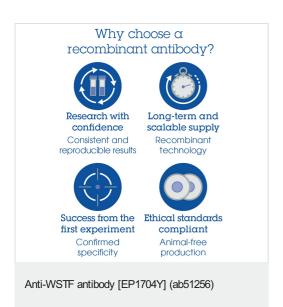
Lane 4 : PC12 cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 171 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab51256 observed at 175 kDa. Red - loading control, <u>ab18058</u>, observed at 124 kDa.

ab51265 was shown to recognize WSTF in wilt-type cells along with additional cross-reactive bands as signal was lost in WSTF knockout samples. Wild-type and WSTF knockout samples were subjected to SDS-PAGE. ab51256 and ab18058 (loading control to vinculin) were diluted 1/15 000 and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



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